ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1995, p. 1247–1252 Vol. 39, No. 6
0066-4804/95/$04.00+0

Clinical Pharmacokinetics of Cidofovir in Human Immunodeficiency Virus-Infected Patients

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Received 2 September 1994/Returned for modification 3 January 1995/Accepted 14 March 1995

The pharmacokinetics of cidofovir (HPMPC; (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine) were examined at five dose levels in three phase I/II studies in a total of 42 human immunodeficiency virus-infected patients (with or without asymptomatic cytomegalovirus infection). Levels of cidofovir in serum following intravenous infusion were dose proportional over the dose range of 1.0 to 10.0 mg/kg of body weight and declined biexponentially with an overall mean ± standard deviation terminal half-life of 2.6 ± 1.2 h ($n = 25$). Approximately 90% of the intravenous dose was recovered unchanged in the urine in 24 h. The overall mean ± standard deviation total clearance of the drug from serum (148 ± 25 ml/h/kg; $n = 25$) approximated renal clearance (129 ± 42 ml/h/kg; $n = 25$), which was significantly higher ($P < 0.001$) than the baseline creatinine clearance in the same patients (83 ± 21 ml/h/kg; $n = 12$). These data indicate that active tubular secretion played a significant role in the clearance of cidofovir. The steady-state volume of distribution of cidofovir was approximately 500 ml/kg, suggesting that the drug was distributed in total body water. Repeated dosing with cidofovir at 3.0 and 10.0 mg/kg/week did not alter the pharmacokinetics of the drug. Concomitant administration of intravenous cidofovir and oral probenecid to hydrated patients had no significant effect on the pharmacokinetics of cidofovir at a 3.0-mg/kg dose. At higher cidofovir doses, probenecid appeared to block tubular secretion of cidofovir and reduce its renal clearance to a level approaching glomerular filtration.

Cidofovir (HPMPC; (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine) is an acyclic nucleotide analog with potent activity against a broad spectrum of herpesviruses, including cytomegalovirus (CMV). The in vivo and in vitro antiviral activities of cidofovir have been reviewed (1). Unlike ganciclovir and other nucleoside analogs currently used for clinical therapy of human herpesvirus infections, cidofovir does not depend on phosphorylation by viral nucleoside kinases to exert its antiviral effect (2). Instead, the drug is phosphorylated to its active form by cellular enzymes. In vitro studies have suggested that the resulting active metabolites are cleared slowly from the intracellular space (2).

Preclinical pharmacokinetic studies with radiolabelled cidofovir in rats and mice (10) and in rabbits and monkeys (3) have demonstrated that the majority of the drug is distributed to the kidneys and is excreted in the urine within 24 h of intravenous administration. In monkeys, a fraction of the radioactive dose (approximately 10%) was excreted in a slow elimination phase, with a terminal elimination half-life of 24 to 35 h. This slower excretion phase may reflect the long intracellular half-life of the phosphorylated metabolites of cidofovir (2). In both monkeys and rabbits, approximately 98% of the excreted radioactive dose was present in the urine as unchanged drug. The oral bioavailability of the drug was estimated to be 3% in rats, 10% in mice, and 23% in monkeys. The dose-limiting toxicity of cidofovir in animals is nephrotoxicity, and its effect on proximal tubular cells has been shown to be ameliorated by concomitant administration of probenecid. In rabbits, concomitant oral probenecid treatment decreased the initial concentration of cidofovir in the cortex of the kidney, while levels in other tissues remained unaffected (3).

In a phase I/II clinical trial with intravenous cidofovir in patients with human immunodeficiency virus (HIV) infection and asymptomatic CMV infection, prolonged and dose-dependent antiviral effects were observed at doses of 3.0 and 10.0 mg/kg of body weight (8). The dose-limiting toxicity was nephrotoxicity, which was dose dependent and was less frequent with concomitant oral probenecid and hydration. This toxicity was characterized by proximal tubular dysfuntion and was consistent with observations in animal toxicity studies. Pharmacokinetic parameters for cidofovir in these patients were dose independent; the mean total clearance of cidofovir from serum was approximately 150 ml/h/kg at both dose levels. This value was significantly higher than the baseline creatinine clearance determined in the same patients, suggesting that active tubular secretion contributed to the renal clearance of cidofovir. There was evidence in two patients of a prolonged phase in the elimination of cidofovir. However, in the majority of patients the drug displayed a terminal elimination half-life of 3 to 4 h. There were no significant changes in the pharmacokinetics of cidofovir over four infusions, suggesting that the drug did not accumulate.

The present report summarizes all available pharmacokinetic data for intravenous cidofovir given to 42 HIV-infected patients in three clinical studies and incorporates data for serum and urine from 15 patients reported previously as part of an account of a phase I/II study of the safety, tolerance, and

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pharmacokinetics of cidofovir (8). In addition, data on the effect of concomitant probenecid treatment on the pharmacokinetics of intravenous cidofovir are presented.

MATERIALS AND METHODS

Patients. All clinical studies were conducted with the informed consent of the patients and the approval of the appropriate institutional review boards. Patients (39 males and 3 females) were selected on the basis of diagnosed HIV infection and normal renal, hepatic, hematologic, and coagulation functions. The mean patient age was 39 years (range, 28 to 55 years), and the median CD4 lymphocyte count was 114 cells per mm^3 (range, 0 to 595 cells per mm^3). Detection of CMV infection in semen, urine, or blood was an eligibility requirement for two of the three clinical studies. CMV was detected following coculture with fibroblasts and was determined qualitatively by observation of cytopathic effects or quantitatively by determination of PFU (5). Patients were entered into the study a maximum of 28 days after CMV was detected. Exclusion criteria included active serious infections (including active CMV disease), clinically significant cardiac disease, pregnancy, previous therapy with ganciclovir or foscarnet, and ongoing therapy with any anti-CMV drugs or agents with nephrotoxic potential. Concomitant therapy with zidovudine (20 patients total) was permitted, and the zidovudine dose was reduced by 50% on the day of cidofovir administration. Since cidofovir is potentially nephrotoxic and didanosine is cleared by renal excretion, concomitant use of didanosine was excluded. Concomitant therapy with zalcitabine (three patients total) was permitted in two of the three studies.

Study design. (i) Drug administration. Cidofovir was formulated as a sterile solution for parenteral administration; the solution contained 25 or 75 mg of cidofovir per ml. Cidofovir was infused in 100 ml of 0.9% (normal) saline into a peripheral vein over a 1-h period. Patients designated for hydration were given an intravenous infusion of 1 liter of 0.9% (normal) saline over 1 h immediately prior to cidofovir administration. Where indicated, probenecid was administered orally as 500-mg tablets. Patients designated to receive probenecid were given either a low-dose or a high-dose regimen. The low-dose regimen consisted of 1 g of probenecid given at 3 h prior to the cidofovir infusion; this was followed by the administration of 500 mg of probenecid each at 2 and 8 h after the end of the cidofovir infusion. The high-dose regimen consisted of 2 g of probenecid given at 3 h prior to the cidofovir infusion; this was followed by the administration of 1 g of probenecid each at 2 and 8 h after the end of the cidofovir infusion.

(ii) Pharmacokinetic studies. Cidofovir was administered to HIV-infected patients at various dose levels in three clinical protocols.

In the first study, conducted on an inpatient basis at the Mount Zion Medical Center of the University of California, cidofovir was administered intravenously to five patients each at doses of 3.0 and 10 mg/kg. The median CD4 cell count was 81 cells per mm^3 (range, 5 to 206 cells per mm^3). Ten milliliters of blood (approximately 6 ml of serum) was withdrawn from each subject at 0 (preinfusion), 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h. Blood was allowed to coagulate and serum was decanted, frozen, and stored at −20°C until it was analyzed. Urine samples were obtained over the intervals 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h postdose, frozen, and maintained at −20°C until they were analyzed. Two patients at each dose level were also given hydration prior to administration of the cidofovir dose. Pharmacokinetics were not evaluated in additional patients given cidofovir at the 0.5- and 1.5-mg/kg doses. Five patients receiving cidofovir at the 3.0-mg/kg dose level received additional doses of cidofovir at 3.0 mg/kg once per week for up to 4 weeks. One patient receiving the drug at the 10.0-mg/kg dose level received three additional doses of cidofovir at 10.0 mg/kg once per week for 4 weeks. Following administration of the fourth dose, serum and urine samples were obtained as described above for the initial dose studies. Data for two patients receiving cidofovir at the 10-mg/kg dose level were excluded from pharmacokinetic calculations because of nephrotoxicity. Five additional patients received cidofovir at a 3.0-mg/kg dose by intravenous infusion, together with hydration and concomitant high-dose probenecid treatment.

In the second study, conducted on a temporary inpatient (>24-h) basis at the Johns Hopkins University School of Medicine, three groups of five patients each received intravenous cidofovir at 1.0-, 3.0-, and 10-mg/kg doses by intravenous administration. The median CD4 cell count was 210 cells per mm^3 (range, 13 to 595 cells per mm^3). Ten milliliters of blood (approximately 6 ml of serum) was withdrawn from each subject at 0 (preinfusion), 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h. Blood was allowed to coagulate and serum was decanted, frozen, and stored at −20°C until it was analyzed. Urine samples were collected prior to dosing and over the intervals 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h postdose.

In the third clinical study, conducted on an outpatient basis at the National Institutes of Health, four groups of patients (two per group) received cidofovir at a 5.0-mg/kg dose by intravenous infusion with various combinations of hydration and/or probenecid. The median CD4 cell count was 62 cells per mm^3 (range, 0 to 182 cells per mm^3). Three patients receiving cidofovir at the 5.0-mg/kg dose with high-dose probenecid received additional infusions once per week for a total of 4 weeks. Four patients received cidofovir at a 7.5-mg/kg dose once every 3 weeks by intravenous infusion for a total of four infusions, together with hydration and high-dose probenecid. Ten milliliters of blood (approximately 6 ml of serum) was withdrawn from each subject at 0 (preinfusion), 1, 2, 3, 4, 8, 12, 24, and 72 h. Blood was allowed to coagulate and serum was decanted, frozen, and stored at −20°C until it was analyzed. Urine samples were collected prior to dosing and over the intervals 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h postdose.

Analytical procedures. (i) Materials. Cidofovir reference standard and the internal standard, 9-(2-phosphonylmethoxyethyl)guanine (PMEG), were synthesized by Gilead Sciences, Inc. (Foster City, Calif.). Potassium phosphate, dibasic (anhydrous), sodium dihydrogen phosphate, and sodium hydroxide were obtained from Mallinckrodt (Paris, Ky.). *o*-Phosphoric acid (85%) was from Fisher Scientific (Fair Lawn, N.J.). Tetrabutylammonium dihydrogen phosphate (TBAHP) was obtained from Fluka Chemical Corp. (Ronkonkoma, N.Y.). Ion pair reagent, 0.5 M octyltriethylammonium phosphate (Q8), acetonitrile, methanol, and deionized water were obtained from Baxter (McGaw Park, Ill.). Pooled normal human serum was obtained from Whittaker (Walkersville, Md.). Pooled normal human urine was obtained from volunteers.

(ii) Determination of cidofovir in serum. Concentrations of cidofovir in clinical serum samples were determined by a validated reverse-phase ion pairing high-performance liquid chromatography (HPLC) method with UV detection. Serum (0.5 ml) was added to 250 μl of internal standard (2.5 μg of PMEG per ml) in a polypropylene centrifuge tube, and the contents were vortexed to mix. Tubes were incubated at 63 ± 2°C for 25 min to inactivate HIV in a Lab-Line Imperial III Incubator (Baxter) and were allowed to cool. Bond Elut SAX ion-exchange solid-phase extraction columns (Jones Chromatography, Lakewood, Colo.) were conditioned with 1.2 ml each of methanol and deionized water. Samples were applied and drained slowly, and the columns were rinsed twice with 0.75 ml of deionized water and air dried. Cidofovir was eluted with 1.0 ml of 60% methanol–40% 100 mM phosphate buffer (pH 2.0). Effluent was neutralized with 0.5 M sodium hydroxide and was evaporated to dryness under reduced pressure. Dried samples were reconstituted in 0.3 ml of mobile phase A (see below) and were transferred to autoinjector vials for HPLC analysis. The HPLC system comprised a model 600E System Controller and a model 715 Ultra Wisp sample processor (Waters Chromatography Division, Milford, Mass.), a model LC-95 UV/visible spectrophotometer detector, and a model 1020 personal integrator (Perkin-Elmer, Norwalk, Conn.). The analytical column was a Zorbax C-8 column (5 μm, 250 by 4.6 mm) equipped with a Zorbax C-8 guard column (6.0 by 40 mm; Mac Mod Analytical Inc., Chadds Ford, Pa.). The mobile phases were 5% acetonitrile and 95% 100 mM phosphate buffer (pH 7.2) containing 5 mM TBAHP (mobile phase A) and 15% acetonitrile and 85% 100 mM phosphate buffer (pH 7.2) containing 5 mM TBAHP (mobile phase B). A linear gradient was used from 100% mobile phase A to 100% mobile phase B over 9 min. The flow rate was 1.4 ml/min, and the column temperature was 40°C. Peaks were detected by determining the UV A_{274}. The retention times on this system were as follows: cidofovir, 6.0 min; PMEG, 8.0 min. The method was linear over the range of 220 to 2,190 ng/ml, and the limit of quantitation was 220 ng/ml. The between-run precision and accuracy were <11 and <3%, respectively, at the limit of quantitation.

(iii) Determination of cidofovir in urine. Concentrations of cidofovir in clinical urine samples were determined by a validated reverse-phase ion pairing HPLC method with UV detection. Urine (0.5 ml) was added to 100 μl of internal standard (250 μg of PMEG per ml in 10 mM phosphate buffer [pH 7.0]) in a polypropylene centrifuge tube, and the contents were vortexed to mix. Tubes were incubated at 63 ± 2°C for 45 min to inactivate virus in a Lab-Line Imperial III Incubator (Baxter) and were allowed to cool. Analytichem Baker Bond C-18 solid-phase extraction columns (J. T. Baker, Phillipsburg, N.J.) were conditioned with one column volume (1 ml) each of methanol and 0.1 N hydrochloric acid. Samples were applied, and effluent (400 μl) was transferred to Ultrafree-ML 5000 NMWL molecular weight cutoff filter units (Millipore Corp., Bedford, Mass.). The filter units were centrifuged for 12 min at 13,000 × *g*. The filtrate (150 μl) was transferred to autoinjector vials for HPLC analysis. The HPLC system was an LC Module-1 comprising a model 600 E system controller, a model 486 UV/visible detector, and a model 715 Ultra Wisp sample processor (Waters Chromatography Division). Data were acquired with Millennium 2010 Chromatography Manager Version II (Waters Chromatography Division). The HPLC column was a Beckman Ultrasphere ODS-IP (150 by 4.6 mm; Alltech, San Jose, Calif.) equipped with a Brownlee RP-18 Newguard Column (15 by 3.2 mm; Alltech, Deerfield, N.Y.). The mobile phase was 5% acetonitrile, 5% methanol, and 90% water containing 5 mM Q8. The flow rate was 2.0 ml/min. The column was rinsed with 25% acetonitrile–25% methanol–50% water containing 5 mM Q8 between runs at 3.0 ml/min. The injection volume was 40 μl, and the column temperature was 40°C. Peaks were detected by determining the UV A_{274}. Retention times were as follows: cidofovir, 6.6 min; PMEG, 8.4 min. The method was linear over the range of 1.0 to 99 μg/ml, and the limit of quantitation was 1.0 μg/ml. The between-run precision and accuracy were <6 and <5%, respectively, at the limit of quantitation.

Pharmacokinetics and statistical analysis. (i) Pharmacokinetic calculations. The pharmacokinetic parameters for intravenous cidofovir were assessed by application of the nonlinear curve-fitting software package PCNONLIN (11) by noncompartmental methods. Pharmacokinetic parameters obtained by compartmental analysis of data from the first clinical study (open two-compartment model) were not significantly different from the results obtained by the noncompartmental method. The parameters estimated by PCNONLIN included the maximum concentration of cidofovir in serum (C_{max}), the time to C_{max}, the area under the serum concentration-versus-time curve up to the time of the last quantifiable concentration ($AUC_{0-t_{last}}$), the value of AUC extrapolated to infinity

($AUC_{0-\infty}$), the slope of the terminal elimination phase (k_e), the half-life of the terminal elimination phase ($0.693/k_e$), the area under the first moment of $AUC_{0-\infty}$, and the mean residence time. A minimum of three datum points was used in the projection of the terminal phase, and the projected area ($AUC_{t_{last}-\infty}$) accounted for an average of <7% of the total area ($AUC_{0-\infty}$). Additional parameters were calculated manually. Total clearance (CL) from serum was calculated as dose/$AUC_{0-\infty}$. The steady-state volume of distribution (V_{ss}) was calculated as mean residence time × CL. The volume of distribution on the basis of area was calculated as CL/k_e.

The cumulative amount of cidofovir excreted at the end of each urine collection period, U_{0-t}, was calculated as the sum of the amounts excreted in all previous collection periods. The cumulative percentage of the dose excreted at the end of each collection period was calculated as $100 \times (U_{0-t}/\text{dose})$. The amount of cidofovir remaining to be excreted was calculated for each time point as $U_{0-24} - U_{0-t}$. This calculation makes the assumption that no further excretion of cidofovir occurs beyond 24 h postdose. This assumption is valid if the total collection period is longer than four elimination half-lives. The slope of a semilogarithmic plot of the amount remaining to be excreted against time provided an estimation of the elimination rate, k_e'. The half-life was then calculated as $0.693/k_e'$. These values (data not shown) were generated to support observations with serum, since the terminal phases often occurred in a concentration range in serum close to the quantitation limit of the analytical method. When not directly available, the concentration of cidofovir in serum at the end of the 24-h urine collection period (C_{24}) was calculated by extrapolation of concentrations in serum as $C_{last} \times e^{-k_e \times (24 - t_{last})}$. The AUC up to the end of the urine collection period (AUC_{0-24}) was calculated as $AUC_{0-\infty} - (C_{24}/k_e)$. The renal clearance ($CL_R$) of cidofovir (in milliliters per hour per kilogram) following intravenous administration was calculated as $(U_{0-24} \cdot 1{,}000)/(AUC_{0-24} \cdot Wt)$, where Wt is the body weight of the patient (in kilograms). Baseline creatinine clearances were determined by direct measurement of creatinine levels in urine.

Statistical comparisons between the first and fourth intravenous doses of cidofovir or between CL_R and baseline creatinine clearances determined in the same patients were performed by a paired *t* test. The effect of probenecid on pharmacokinetic parameters for cidofovir was evaluated at the 3-mg/kg cidofovir dose by an unpaired *t* test. The effect of probenecid was also examined by unpaired comparison of data for all patients given cidofovir alone with data for patients given 5 or 7.5 mg of cidofovir per kg with high-dose probenecid and hydration. A *P* value of <0.05 was considered significant.

(ii) Protein binding. Binding of cidofovir to plasma or serum proteins was evaluated over the concentration range of 0.25 to 25.0 μg/ml by using ^{14}C-labelled cidofovir in pooled normal human plasma or serum. Duplicate samples were incubated at 37°C for 20 min and were centrifuged through Ultrafree 10,000-molecular-weight-cutoff filters (Millipore) in a heated centrifuge (approximately 32°C). Results were corrected for nonspecific binding by comparison with recovery from buffer. Binding of cidofovir to protein was negligible (<0.5%) over the entire concentration range.

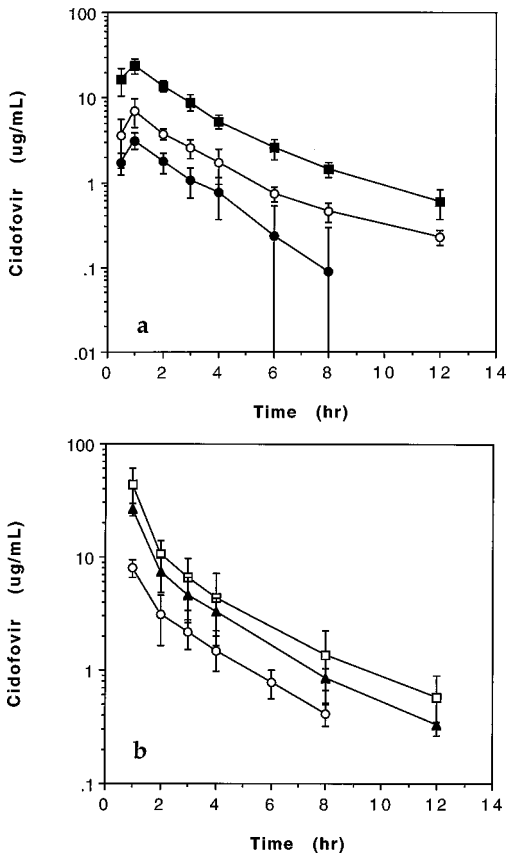

FIG. 1. Effect of dose on mean ± SD concentrations of cidofovir following intravenous infusion in HIV-infected patients. (a) Cidofovir alone; ●, 1.0 mg/kg (*n* = 5); ○, 3.0 mg/kg (*n* = 10); ■, 10.0 mg/kg (*n* = 8). (b) Cidofovir with concomitant hydration and high-dose oral probenecid: ○, 3.0 mg/kg (*n* = 5); ▲, 5.0 mg/kg (*n* = 3); □, 7.5 mg/kg (*n* = 4).

RESULTS

Initial dose studies. Figure 1a compares the mean ± standard deviation (SD) concentrations of cidofovir in serum following the initial intravenous administration to HIV-infected patients at three dose levels; the corresponding appearance of cidofovir in urine is shown in Fig. 2a. By using the current analytical methods, no metabolites of cidofovir were observed in any of the serum or urine samples analyzed. Concentrations in serum declined biexponentially, with an overall mean terminal half-life of 2.6 ± 1.2 h (*n* = 25). Maximum concentrations of cidofovir in serum following intravenous infusion increased proportionally with dose (Fig. 3).

Table 1 summarizes the noncompartmental pharmacokinetic parameters for intravenous cidofovir over the dose range of 1.0 to 10.0 mg/kg and the overall mean parameters for 25 patients given cidofovir by the intravenous route. The observed AUCs were dose proportional. The overall mean ± SD recovery of unchanged cidofovir in urine following administration of an intravenous dose was 90.3% ± 27.0% (*n* = 25). The overall mean CL of the drug from serum (148 ± 25 ml/h/kg; *n* = 25) approximated CL_R (129 ± 42 ml/h/kg; *n* = 25), which was significantly higher ($P < 0.001$) than the baseline creatinine clearance determined in the same patients prior to cidofovir administration (83 ± 21 ml/h/kg; *n* = 12). The terminal elimination half-lives at the 3.0- and 10.0-mg/kg dose levels were equivalent, while the half-life at the 1.0-mg/kg dose level was significantly shorter. This may have been an artifact produced by an inability to determine levels in serum very close to the limit of quantitation of the analytical method at the lowest dose. Pharmacokinetic parameters were not calculated for two additional patients given 10 mg of cidofovir per kg since the elevated creatinine levels observed in the sera of these patients suggested that the CL_R of the drug would have been altered.

Multiple-dose studies. Table 1 compares the major pharmacokinetic parameters for the first and fourth doses of cidofovir at the 3.0-mg/kg/week dosage level. Repeated dosing with cidofovir did not significantly alter the pharmacokinetic parameters of the drug ($P > 0.415$). Data were available for a fourth infusion in a single patient at the 10.0-mg/kg/week dosage level (data not shown), since only one of five patients tolerated four dosages at this level.

Effect of concomitant probenecid. The concentrations of cidofovir in serum following intravenous infusion at a 5.0-mg/kg dose with various combinations of hydration and probenecid were similar for all treatments, with the exception that the concentration in serum at the end of infusion was elevated in patients given hydration and high-dose probenecid. The mean C_{max} values were 11.6 μg/ml (*n* = 2), 12.5 μg/ml (*n* = 2), 26.1 ± 3.2 μg/ml (*n* = 3), and 15.0 μg/ml (*n* = 2) for hydration alone, hydration with low-dose probenecid, hydration with high-dose probenecid, and high-dose probenecid alone, respectively. The low-dose regimen of probenecid did not appear to affect the pharmacokinetics of cidofovir.

Figure 1b compares the mean ± SD concentrations of cido-

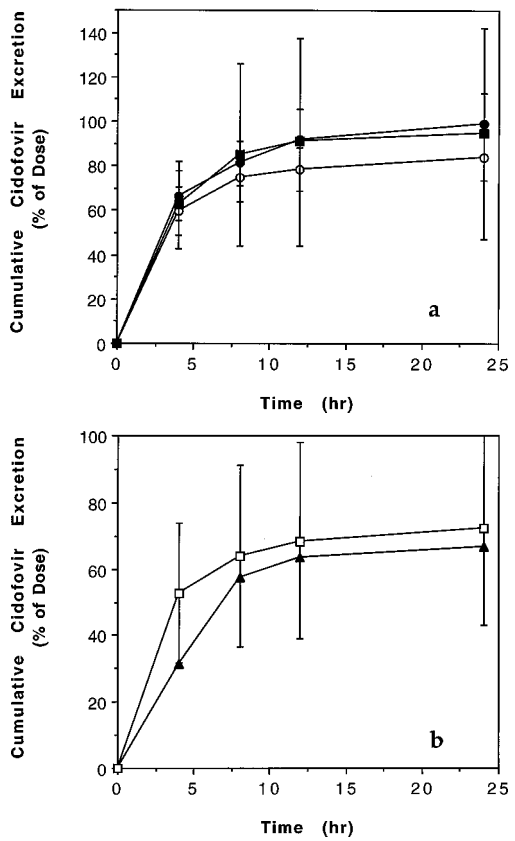

FIG. 2. Urinary excretion of cidofovir following intravenous administration to HIV-infected patients (mean ± SD). (a) Cidofovir alone; ●, 1.0 mg/kg (n = 5); ○, 3.0 mg/kg (n = 5); ■, 10.0 mg/kg (n = 5). (b) Cidofovir with concomitant hydration and high-dose oral probenecid: ▲, 5.0 mg/kg (n = 2); □, 7.5 mg/kg (n = 3).

fovir in serum following administration at three intravenous dose levels to hydrated patients with concomitant high-dose oral probenecid. The corresponding recovery of cidofovir in urine is shown in Fig. 2b. Table 2 summarizes the mean noncompartmental pharmacokinetic parameters determined for the three dose levels and the overall mean data for all 12 patients given cidofovir with hydration and high-dose probenecid. The levels of cidofovir in serum declined in an apparent biexponential manner, with a mean ± SD terminal half-life of 2.4 ± 0.5 h (n = 12). This value was not significantly different from the half-life of elimination of cidofovir in the absence of probenecid (P = 0.545). Probenecid had no apparent effect on the pharmacokinetics of cidofovir at a 3.0-mg/kg dose. However, at the 5.0- and 7.5-mg/kg dose levels, probenecid significantly decreased the CL (P = 0.002) and the V_{ss} (P = 0.001) of cidofovir.

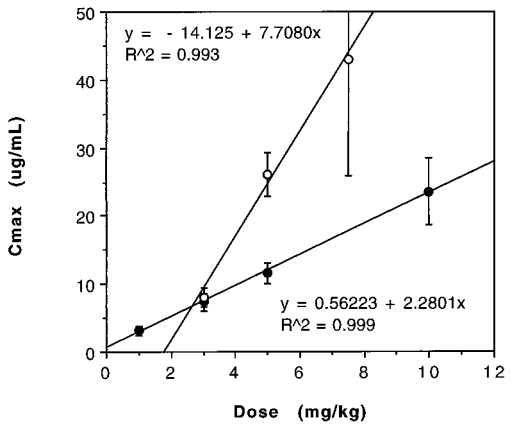

FIG. 3. Relationship between cidofovir dose and the mean ± SD maximum concentration achieved in serum following intravenous infusion without probenecid (●) and with high-dose probenecid (○).

Following intravenous administration of cidofovir with concomitant high-dose probenecid and hydration, the resulting C_{max} and AUC values appeared to deviate from dose proportionality (Fig. 3). The overall mean ± SD recovery of unchanged cidofovir in the urine of these patients was 70.1% ± 21.4% (n = 5). The overall mean CL of the drug from serum (125 ± 38 ml/h/kg; n = 12) was significantly higher than CL_R (82 ± 40 ml/h/kg; n = 5) (P = 0.040, on the basis of a paired t test), while the latter was close to the baseline creatinine clearance determined in the same patients. At a 5-mg/kg dose of cidofovir, concomitant high-dose probenecid appeared to reduce the CL_R of cidofovir to the level of glomerular filtration, presumably by blocking the active tubular secretion of the drug. The mean CL_R of cidofovir in patients receiving concomitant high-dose probenecid alone was 110 ml/h/kg (n = 2), compared with a mean baseline creatinine clearance of 64 ml/h/kg (n = 2) in the same patients.

There was no evidence of a change in the pharmacokinetics of cidofovir over four infusions at 5 mg/kg/week or three infusions at 7.5 mg/kg every 3 weeks when it was coadministered with probenecid and hydration (data not shown).

DISCUSSION

Following intravenous administration, the pharmacokinetics of cidofovir were characterized by an apparent biexponential decline in concentrations in serum, with an elimination half-life of approximately 2.5 h. The majority of the administered drug was recovered unchanged in the urine, and no metabolites of cidofovir were detected in clinical urine or serum samples by the current analytical methods. No evidence of drug accumulation was seen when cidofovir was administered as a once-per-week infusion. The relatively short half-life of cidofovir may not reflect the true duration of action of the drug, since the antiviral effect is dependent on concentrations of the active phosphorylated metabolites of cidofovir present within the cell. The limitations of the current analytical methods may preclude observation of a prolonged terminal elimination phase representing efflux of cidofovir from cells. Such a prolonged phase has been observed in preclinical studies with radiolabelled drug (3).

In the absence of probenecid, the V_{ss} of cidofovir was approximately 500 ml/kg. This V_{ss} is very similar to those reported for stavudine and zalcitabine (6), suggesting that cidofovir was distributed in total body water. At cidofovir doses of 5 mg/kg or greater, this volume was significantly reduced by concomitant administration of high-dose probenecid (P = 0.001), suggesting competition for an anion uptake mechanism. Concomitant probenecid treatment decreases the volumes of distribution of numerous drugs, and the possibility of decreased efficacy as a result of lower levels in tissue has been proposed (4). However, preclinical studies in rabbits have not shown a significant effect of probenecid on the levels of cidofovir in tissue other than in the kidney (3). Potential interac-

TABLE 1. Pharmacokinetic parameters for cidofovir after administration of the first intravenous dose to HIV-infected patients[a]

Dose (mg/kg)	No. of subjects	Wt (kg)	C_{max} (μg/ml)	$AUC_{0-\infty}$ (μg · h/ml)	CL (ml/h/kg)	CL_R (ml/h/kg)	CL_{CR} (ml/h/kg)	V_{area} (ml/kg)	MRT (h)	V_{ss} (ml/kg)	$t_{1/2}$ (h)	% Recovery in urine at 24 h
1.0	5	89.2 (23.8)	3.12 (0.67)	8.35 (3.10)	130 (37)	129 (38)		257 (148)	2.7 (0.7)	339 (74)	1.44 (0.87)	98.5 (14.1)
3.0	10	76.5 (4.8)	7.34 (1.39)	19.96 (2.30)	152 (18)	129 (23)	73 (14)[b]	592 (176)	3.5 (0.9)	533 (135)	2.72 (0.82)	83.9 (10.3)
5.0	2	84.3	11.5	28.34	177	149	99	609	3.2	556	2.42	85.9
10	8	67.3 (12.3)	23.56 (4.88)	68.80 (9.48)	148 (20)	124 (66)	89 (31)[c]	663 (312)	3.5 (0.7)	516 (119)	3.14 (1.44)	94.7 (47.5)
Mean	25	76.7 (14.8)			148 (25)	129 (42)	83 (21)[d]	549 (261)	3.3 (0.8)	490 (136)	2.57 (1.88)	90.3 (27.0)
3.0[e]	5		7.93 (1.71)	21.4 (4.7)	146 (31)	119 (24)		818 (461)	5.6 (3.6)	795 (539)	4.1 (2.8)	81.0 (4.3)

[a] Values are means (SDs). Abbreviations: CL_{CR}, creatinine clearance; V_{area}, volume of distribution on the basis of area; MRT, mean residence time; $t_{1/2}$, terminal-phase half-life; the other abbreviations are defined in the text.
[b] Mean of five patients only.
[c] Mean of three patients only.
[d] Mean of 10 patients only.
[e] Infusion 4.

tions of probenecid with other drugs in a clinical setting may require dose reduction on the day of cidofovir administration.

The mechanism of the nephrotoxicity of cidofovir observed in animal models in unknown, but the effect is apparently alleviated by concomitant administration of probenecid, a known inhibitor of the active tubular secretion of acidic drug molecules (4). As such, it was postulated that the nephrotoxicity of cidofovir may be directly related to its active tubular secretion in the kidney. Very high concentrations of drug have been detected in the kidneys of animals given intravenous cidofovir (3), and the initial concentrations were reduced by concomitant probenecid treatment. This suggests that initial transport of cidofovir into proximal tubular cells across the basolateral membrane was faster than efflux into urine.

In clinical studies, baseline creatinine clearances, determined in patients prior to administration of the first cidofovir dose, were used as a measure of the glomerular filtration rate. In the absence of probenecid, the CL of cidofovir was attributed entirely to renal excretion, and the CL_R of cidofovir was consistently higher than baseline creatinine clearances in the same patients. The excess CL of cidofovir above the level of glomerular filtration must have been a consequence of active tubular secretion of the drug in the kidney. Similar renal tubular secretion has been reported for structurally related antiviral nucleoside analogs, including stavudine (6) and didanosine (7).

A low-dose regimen of probenecid did not appear to alter the pharmacokinetics of cidofovir. The selective elevation of C_{max} by concomitant hydration and high-dose probenecid treatment may have been the result of a transient decrease in the volume of distribution of cidofovir produced by high levels of probenecid. The cellular mechanism responsible for the inhibition of renal tubular transport by probenecid is not known (4). Data on the pharmacokinetics of cidofovir in serum and recovery in urine suggest that probenecid decreased the CL_R of cidofovir by competitive inhibition of the active tubular secretion pathway in the proximal tubules of the kidney. This may be supported by the lack of an apparent effect of low-dose probenecid. However, a simple competitive mechanism does not explain the lack of an effect of high-dose probenecid on lower doses of cidofovir (3.0 mg/kg). One obvious explanation would be a failure to detect a difference at this dose because of a high degree of variability in the data. This explanation is not supported by the very consistent data available for a total of 15 patients who received this dose.

An alternative explanation for the data is the possible existence of multiple secretion pathways for cidofovir, including a pathway not subject to inhibition by probenecid. At low cidofovir doses, the drug may be cleared from the serum by this high-affinity transport system, while at higher cidofovir doses, this system may be saturated, requiring cidofovir to be transported by a probenecid-sensitive mechanism. Since the available data are somewhat limited, such a hypothesis remains speculative. However, there is a precedent for multiple tubular secretion pathways, including a probenecid-insensitive system, in the renal clearance of diprophylline, a dihydroxypropyl analog of theophylline (9). It is therefore possible that a probenecid-insensitive transport system specific to purines and pyrimidines exists in the proximal tubule.

In summary, the pharmacokinetics of intravenous cidofovir in HIV-infected patients were reproducible and dose independent. Systemic exposure to the drug was proportional to the intravenous dose. The drug was cleared by the kidney and was excreted extensively as unchanged cidofovir in the urine. The observed rate of urinary excretion of cidofovir may not reflect the true duration of action of the drug, but it remains an

TABLE 2. Pharmacokinetic parameters for cidofovir after administration of the first intravenous dose with concomitant high-dose oral probenecid and hydration to HIV-infected patients[a]

Dose (mg/kg)	No. of subjects	Wt (kg)	C_{max} (μg/ml)	$AUC_{0-\infty}$ (μg · h/ml)	CL (ml/h/kg)	CL_R (ml/h/kg)	CL_{CR} (ml/h/kg)	V_{area} (ml/kg)	MRT (h)	V_{ss} (ml/kg)	$t_{1/2}$ (h)	% Recovery in urine at 24 h
3.0	5	70.6 (7.0)	8.08 (1.39)	19.87 (2.85)	154 (23)			488 (171)	3.0 (0.2)	457 (75)	2.2 (0.6)	
5.0	3	71.5 (1.9)	26.07 (3.24)	50.56 (6.63)	100 (12)	72[b]	91[b]	322 (83)	2.6 (0.2)	261 (19)	2.2 (0.5)	66.7[b]
7.5	4	69.6 (13.4)	42.95 (17.09)	79.67 (32.23)	107 (45)	90 (55)[c]		947 (773)	2.8 (0.4)	301 (151)	2.6 (0.4)	72.4 (29.4)[c]
Mean	12	70.6 (7.5)			125 (38)	82 (40)[d]	91[b]	600 (495)	2.8 (0.3)	356 (129)	2.3 (0.5)	70.1 (21.4)[d]

[a] Values are means (SDs). See text and footnote *a* of Table 1 for definitions of abbreviations.
[b] Mean of two patients.
[c] Mean (SD) of three patients.
[d] Mean (SD) of five patients.

important parameter in the management of potential nephrotoxicity. Concomitant oral probenecid decreased the CL_R of cidofovir, presumably by blocking its active tubular secretion. This observation provides further support for the clinical use of concomitant probenecid as a nephroprotectant during cidofovir therapy.

ACKNOWLEDGMENT

We gratefully acknowledge the staff of Nichols Institute Laboratories, San Juan Capistrano, Calif., for technical assistance.

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